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Why the Epidermal Growth Factor Receptor? The Rationale for Cancer Therapy

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Explain the molecular biology of epidermal growth factor receptor (EGFR) function in malignant cells.
2. Recognize the relationships between and functions of the erbB family of related cell membrane receptors.
3. Describe the current status of clinical strategies to inhibit EGFR function in malignant cells.

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ABSTRACT

There is a need for new, selective anticancer agents that differentiate between malignant and nonmalignant cells. The benefits of such agents would include a higher therapeutic index and lower toxicity than conventional therapies. Although expressed in nonmalignant cells, the epidermal growth factor receptor (EGFR) is highly expressed in a variety of tumors, and its expression correlates with poor response to treatment, disease progression, and poor survival. Evidence for a role for the EGFR in the inhibition and pathogenesis of various cancers has led to the rational design and development of agents that selectively target this receptor. Activation of the EGFR signaling pathway in cancer cells has been linked with increased cell proliferation, angiogenesis, and metastasis, and decreased apoptosis. Preclinical data show that anti-EGFR therapies can inhibit these effects in vitro and in vivo. In addition, preclinical data confirm that many such agents have the potential to increase the effectiveness of current cytotoxic agents. Following accelerated drug development programs, phase III trials are now under way for a number of EGFR-targeted therapies, including the monoclonal antibody IMC-C225 and the EGFR-tyrosine kinase inhibitors ZD1839 (Iressa™) and OSI-774. Thus, the rationale for EGFR-targeted approaches to cancer treatment is apparent and now well established, and there is increasing evidence that they may represent a significant contribution to cancer therapy. *The Oncologist 2002;7(suppl 4):2-8*

INTRODUCTION

Over the past few decades, there has been considerable interest in developing new agents to improve the outcome for patients with solid tumors. However, traditional cytotoxic therapies are nonspecific and do not discriminate between tumor and host cells [1]. Further, as they are generally effective against rapidly dividing neoplasms [2], their efficacy against solid tumors is limited. Even where cytotoxic agents are effective, tumor resistance may develop [3]. The lack of specificity and limited efficacy of traditional

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cytotoxic agents has led to the rational design and development of targeted therapies that aim to differentiate between malignant and nonmalignant cells, thereby producing a higher therapeutic index and less toxicity than conventional therapies [1]. In order to develop such agents, it is necessary to identify the aberrant biochemical and molecular pathways that distinguish malignant cells from nonmalignant cells [2]. As with nonmalignant cells, tumor growth and progression depend largely on the activity of cell membrane receptors that control the intracellular signal transduction pathways regulating cell proliferation and apoptosis, angiogenesis, adhesion, and motility [2].

One such cell membrane receptor is the epidermal growth factor receptor (EGFR), which has been shown to play an important role in the growth and survival of many solid tumors. Pathways involved in EGFR signal transduction have been proposed as possible anticancer targets, and agents to specifically target the EGFR have been developed [4–6].

EGFR and Signaling Pathways

The EGFR belongs to the erbB family of four closely related cell membrane receptors: EGFR (HER1 or erbB1), erbB2 (HER2), erbB3 (HER3), and erbB4 (HER4). These receptors are transmembrane glycoproteins that consist of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity for signal transduction (Fig. 1). Activation of the EGFR occurs when a ligand, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), or amphiregulin, binds to its extracellular domain. This causes the receptor to dimerize with either another EGFR monomer or with another member of the erbB family [7]. Following receptor dimerization, activation of the intrinsic protein tyrosine kinase activity and tyrosine autophosphorylation occur. These events lead to the recruitment and phosphorylation of several intracellular substrates, leading to mitogenic signaling and other cellular activities [8, 9]. Receptors that lack kinase function, because of mutations at the ATP binding site, do not display a full range of biochemical responses following ligand binding [10]; this demonstrates that receptor tyrosine kinase activity is required in cellular signaling. A major signaling route of the erbB family appears to be the *ras-raf*-mitogen-activated protein kinase pathway [8]. Another important pathway in erbB receptor signaling is the one constituted by phosphatidylinositol 3-kinase and the downstream protein kinase Akt [11, 12]. After its activation, Akt transduces signals that regulate multiple biological processes including apoptosis, gene expression, and cellular proliferation [13]. Akt is likely to send survival (antiapoptotic) signals by phosphorylating multiple targets, including the Bcl-2 family member BAD (a proapoptotic factor) [14] and the cell-death pathway enzyme caspase-9 [15]. Akt also plays a prominent role in regulation of cell cycle progression [13]. Thus, EGFR signaling can lead to a variety of downstream reactions, which are subject to complex regulatory mechanisms [16].

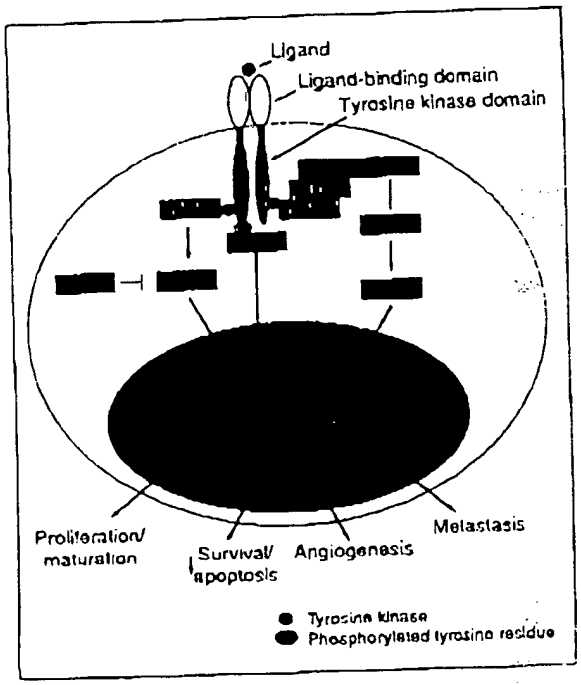

Figure 1. EGFR signal transduction. Adapted from [4] by permission from Signal 2000;1:12-21. ©2000 Adis International Ltd.

EGFR and Cancer

EGFR signaling impacts on many aspects of tumor biology. Activation of the EGFR has been shown to enhance processes responsible for tumor growth and progression, including the promotion of proliferation, angiogenesis, and invasion/metastasis, and inhibition of apoptosis (Fig. 1) [4, 17, 18]. The expression of EGFR in tumors has been correlated with disease progression, poor survival, poor response to therapy [19], and the development of resistance to cytotoxic agents [20, 21]. High levels of EGFR have been observed in a variety of tumors, including prostate, breast, gastric, colorectal, and ovarian [4, 17, 22]. However, mechanisms other than EGFR expression affect EGFR signaling (reviewed by *Arteaga* pp. 31-37 [23]). For example, mutations in the EGFR are observed in some tumors; the most common mutant is EGFRvIII, which lacks an external ligand-binding domain and has a constitutively activated, but attenuated, tyrosine kinase [17]. EGFRvIII is commonly overexpressed as a result of gene amplification and has been identified in brain, lung, breast, prostate, and stomach cancers [6] but has not yet been found in nonmalignant cells.

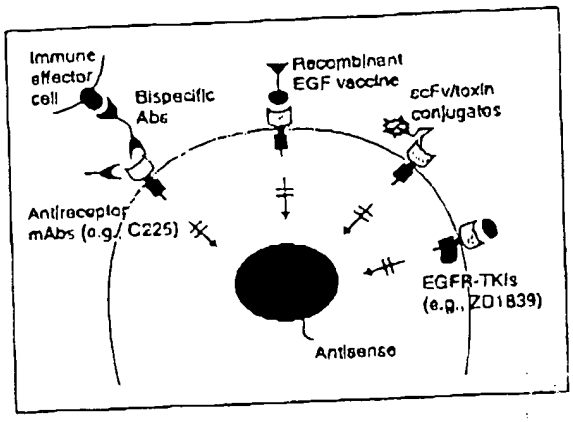

Figure 2. Strategies for EGF signaling inhibition. Adapted from [6] by permission from Drugs 2000;60(suppl 1):15-23. ©2000 Adis International Ltd. Abbreviations: EGF = epidermal growth factor; EGFR-TKI = epidermal growth factor receptor tyrosine kinase inhibitor; scFv = single-chain fragment variable.

Current EGFR-Targeted Strategies

The clear potential for EGFR-targeted therapies in the treatment of cancer has prompted the development of a variety of agents targeted to the extracellular ligand-binding domain, the intracellular tyrosine kinase domain, the ligand, or to synthesis of the EGFR (Fig. 2). These agents are being investigated as monotherapy as well as in combination with conventional therapies.

A number of monoclonal antibodies (mAbs) directed against the extracellular ligand-binding domain, which prevent ligand binding (e.g., IMC-C225 and ABX-EGF), have been developed. Another approach is provided by bispecific antibodies (e.g., MDX-447) that target the extracellular ligand-binding domain of the EGFR as well as epitopes on the surface of immune effector cells, such as macrophage-activated killer cells. The aim is to encourage immune effector cell recruitment at the site of tumors, and hence, initiate destruction of the tumor cells and then stimulation of additional immune responses. Single-chain fragment variable (scFv) antibodies against the EGFR conjugated to toxins, such as pseudomonas endotoxin A (ETA), as well as to fungal and plant-derived toxins, have also been investigated [24]. One of the most potent conjugates is the scFv-14e1-ETA-fusion toxin, which binds to EGFR and EGFRvIII with equal affinity but has 100-fold enhanced cytotoxicity against tumors expressing EGFRvIII compared with those expressing EGFR [25, 26].

Another approach has been to target the intracellular tyrosine kinase domain of the EGFR using small-molecule EGFR tyrosine kinase inhibitors (EGFR-TKIs), such as ZD1839 (Iressa™) and OSI-774. These inhibit ATP binding to the tyrosine kinase domain of the receptor, thereby inhibiting tyrosine kinase activity and autophosphorylation, and subsequently, blocking signal transduction from the EGFR.

Additional tactics used to target EGFR signaling have been directed against its ligands, such as the recombinant EGF vaccine, EGF-P64k, which consists of recombinant human EGF conjugated to a highly immunogenic recombinant bacterial protein P64k [27]. Therapies that target both ligand and EGFR production are also being investigated using antisense oligonucleotides to block the translation of the TGF-α and EGFR genes into their respective proteins.

Of these EGFR-targeted agents, the mAb IMC-C225 and the EGFR-TKIs ZD1839 and OSI-774 are the furthest developed (Table 1). Preclinical data from these agents support the rationale for the use of EGFR-targeted agents in cancer therapy, and their potential is being evaluated in clinical trials.

EGFR-Targeted Agents in Cancer: Preclinical Validation

Both in vitro and in vivo studies have demonstrated that EGFR-targeted agents inhibit the processes involved in tumor growth and progression, including proliferation, apoptosis, metastasis, and angiogenesis. To illustrate the rationale for targeting the EGFR, two agents that operate using different mechanisms are described below: the mAb IMC-C225 and the EGFR-TKI ZD1839.

Table 1. EGFR-targeted strategies and their development stages

Class of compound	Name	Development stage
mAbs	IMC-C225	phase III
	ABX-EGF	phase II
	EMD-72000	phase II
	TheraCIM-h-R3	phase II
	mAb-806	preclinical
Bispecific antibodies	MDX-447	phase II
EGFR-TKIs		
Quinazolines	ZD1839	phase III
	OSI-774	phase III
	CI-1033	phase II
	EKB-569	phase I
	PD-0183805	phase I
Pyridopyrimidines	PD-158780 series	preclinical
	PD-180970	preclinical
Pyrrolopyrimidines	PKI-166	phase I
Other compounds	GW-572016/GW-2016	phase I
	LFM-A12	preclinical
Recombinant vaccine	EGF-P64k	phase II
Antisense oligonucleotides	AS-21	preclinical

The anti-EGFR mAb IMC-C225 (cetuximab) has been shown to inhibit cell growth and survival in vitro and in vivo [28]. It causes an increase in the expression of the cell cycle inhibitor $p27^{KIP1}$, resulting in the formation of inhibitory $p27^{KIP1}$-cyclin-dependent kinase-2 complexes that prevent cells from exiting the G_1 phase of the cell cycle [29]. IMC-C225 has also been shown to induce apoptosis in some cell lines [30] and to inhibit the production of angiogenic factors, in vitro and in vivo [31], as well as metastasis [32].

Data from in vitro studies have revealed that, in addition to reducing cell proliferation, the EGFR-TKI ZD1839 induces cell cycle arrest, increases apoptosis, and has anti-angiogenic activity [3, 33]. In addition, ZD1839 has been shown to have antimetastatic properties in human head and neck and breast cancer cells [34]. ZD1839 inhibited cancer cell migration and invasiveness by blocking p21-activated kinase 1, which is vital for directional motility and cell survival. In vivo studies have confirmed its ability to inhibit tumor growth in a variety of tumor types including prostate, breast, ovarian, colon, small-cell lung, and non-small cell lung cancer (NSCLC) [33, 35, 36]. However, the level of expression of the EGFR in xenografts does not seem to influence the effect of ZD1839, indicating that the level of expression of the EGFR is not the only factor to influence EGFR signaling [37].

Preclinical studies have also demonstrated that EGFR-targeted agents have potential for use in combination with cytotoxic chemotherapy and with radiotherapy. IMC-C225 has been shown to enhance the effects of cytotoxic agents [38-40] and radiotherapy [41, 42]; for example, IMC-C225 in combination with topotecan increased survival of nude mice bearing human colon cancer xenografts [39]. ZD1839 also potentiated the growth-inhibitory effects of cytotoxic agents [35, 36, 43, 44], and preliminary data indicate additive or synergistic effects in combination with ionizing radiation [43].

EGFR-Targeted Agents in Cancer: Clinical Validation

The use of mAbs against the erbB family of receptors has been validated with trastuzumab, a humanized mAb raised against the extracellular domain of erbB2, which gained U.S. Food and Drug Administration (FDA) approval in September 1998 for the treatment of metastatic breast cancer. Trastuzumab is generally well tolerated, although serious cardiac side effects may occur in some patients [45], especially those aged over 60 years or those receiving concomitant doxorubicin/ cyclophosphamide. The cardiotoxicity of trastuzumab is currently not well understood and is under intense scrutiny; it may be related to cardiac expression of erbB2 [46].

The most advanced anti-EGFR mAb in clinical development is IMC-C225. The recently reported preliminary results of the following trials have been promising: a phase III trial of IMC-C225 in combination with cisplatin in patients with metastatic or recurrent head and neck cancer [47]; a phase II trial of IMC-C225 monotherapy in colorectal cancer [48]; and phase I/II trials of combination therapy with cisplatin [49] or cisplatin/carboplatin [50] in squamous-cell carcinoma of the head and neck and with irinotecan/5-fluorouracil/leucovorin in colorectal cancer [51]. The most common adverse event related to IMC-C225 was acneiform rash.

Although the chimeric antibody IMC-C225, formed by replacing the constant region of the original mouse mAb with the constant region of a human immunoglobulin, greatly reduces immunogenicity compared with the original mouse mAb, anaphylactic reactions and loss of efficacy have been seen after repeated exposure, due to the formation of human-antimouse antibodies [46]. A humanized version of IMC-C225, EMD-72000, has, therefore, been developed and, following promising efficacy as a single agent in phase I trials, is under evaluation in phase II trials in patients with ovarian and head and neck cancers [52].

The potential of therapies targeting tyrosine kinases has been demonstrated with imatinib, an inhibitor of tyrosine kinases associated with Bcr-Abl and c-kit and the platelet-derived growth factor receptor tyrosine kinase. Imatinib was launched in the U.S. in May 2001 for the treatment of patients with chronic myeloid leukemia. In February 2002, imatinib gained FDA approval for use in patients with inoperable and/or metastatic malignant gastrointestinal stromal tumors.

Clinical trials have shown that ZD1839 is active in solid tumors, with the most common side effects being mild, reversible rash and diarrhea (reviewed by *Herbst* [53], *Natale* [54], and *Ranson* [55] in this issue). ZD1839 is currently undergoing phase III evaluation in combination with other cytotoxic agents in NSCLC, having demonstrated clinically meaningful activity in phase II trials in patients with head and neck cancer [56] and NSCLC [57, 58], reviewed by *Herbst* in this issue [53]. In addition to the expected antiproliferative effect of EGFR-TKIs, resulting in disease stabilization, partial responses were observed in some patients. Phase I trials have also shown that the combination of ZD1839 with other cytotoxic agents is feasible [59, 60], reviewed by *Ranson* in this issue [55].

Early trials suggest that OSI-774 monotherapy has some activity in NSCLC, head and neck cancer, and ovarian carcinoma [61-63]; combination studies are also under way [64, 65]. Preliminary phase I studies of OSI-774 in combination with standard chemotherapeutic agents, such

as docetaxel, gemcitabine plus cisplatin, and carboplatin plus paclitaxel, have shown no major interactions among OSI-774 and these drugs [64, 66]. A phase III study of OSI-774 with gemcitabine plus cisplatin, using the drug regimen determined in phase I trials, is currently ongoing in Europe in patients with NSCLC [66], and a similar study with carboplatin and taxel is under way in the U.S.

Conclusion

As the EGFR is highly expressed in a variety of solid tumors and is associated with poor response to treatment, disease progression, and poor survival, EGFR inhibition is a logical anticancer strategy. Many potential points of intervention on the receptor have been identified, and mechanisms include inhibition of ligand binding and intracellular signaling. Preclinical results from a multitude of novel anti-EGFR agents have shown that many of the approaches to inhibit EGFR signaling are feasible and that inhibition of the EGFR causes cancer cell proliferation, angiogenesis, and metastasis to decrease, and apoptosis to increase. Many of the intracellular pathways involved with these anticancer effects are being probed; this understanding has led to the development of many agents, potentially benefiting patients with a variety of tumors. The clinically furthest developed: IMC-C225, ZD1839, and OSI-774 are currently undergoing phase III evaluation. In addition to efficacy as monotherapy, these agents successfully enhance the activity of conventional cytotoxic agents and may provide alternative treatment regimens to patients with solid tumors.

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